THE ORIGIN OF THE DIABETOGENIC HORMONE IN THE DOGFISH [1]

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It is generally regarded that the anterior lobe of the pituitary produces a diabetogenic hormone. A good deal of the evidence for this view has been obtained by injection of extracts of the various lobes of the pituitary into normal or hypophysectomized-depancreatized animals. Although posterior and intermediate lobe extracts have been found to be mildly diabetogenic, most of the diabetogenic activity is found in the extracts of the anterior lobe (Houssay, 1937). Recently, an attractive possibility has been presented by O'Donovan and Collip (1938) to the effect that the diabetogenic, ketogenic, and specific metabolic effects of pituitary extracts represent the action of the melanophore hormone, intermedin. This view not only tends to reduce the number of hypophyseal principles but also supplies a function for the melanophore hormone in warm-blooded animals. The evidence for this view is mainly of a chemical nature, since extracts prepared from the pituitary in various ways, or from the intermediate lobe alone, produce the various metabolic effects mentioned above and at the same time are rich in the melanophore hormone.

If intermedin is responsible for the diabetogenic effects, it should be possible to obtain supporting evidence by physiological methods in some cold-blooded vertebrate that depends on the pituitary for the regulation of its integumentary melanophores. A very favorable animal for such an investigation is the smooth dogfish, *Mustelus canis*, in which Orias (1932) has already demonstrated the amelioration of pancreatic diabetes by complete hypophysectomy. The physiology of the intermediate lobe of the pituitary of this animal is well understood, and its secretion can be controlled at will (cf. Abramowitz, 1939*a*). The effects of the presence or absence of intermedin in the blood on the resting blood sugar, or on the severity of experimental diabetes, could then easily be determined. Of considerable interest in this connection is the report of Slome (1936) that the blood sugar of black-adapted toads is significantly higher than

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that of white-adapted toads, a situation which is not unfavorable to the view that intermedin is diabetogenic. However, Slome concluded that the diabetogenic hormone was produced by the anterior lobe since removal of the entire pituitary or of the anterior lobe alone decreased the severity of pancreatic diabetes. Another advantageous feature in using the dogfish is the facility of surgical treatment of the pituitary. Either the anterior or the neuro-intermediate lobe can be removed separately, and it is therefore possible to determine the relative effects of different parts of the hypophysis on carbohydrate metabolism.

The experiments to be described in this paper were undertaken to determine whether intermedin is identical with the diabetogenic hormone. The richness of intermedin in partly purified extracts possessing the ability to produce metabolic effects is suggestive but rather circumstantial evidence that such effects are due to intermedin, since many chemically similar hormones may be present in partly refined preparations. This question can be tested directly in the dogfish by experimental regulation of intermedin secretion. If the chromatic function of the pituitary can be correlated with its diabetogenic function, the probability that both effects are due to the same hormone is enhanced. On the other hand, if these two effects can be shown to occur independently, it becomes reasonably certain that they are not due to the same principle.

Materials and Methods

Pups, yearlings, and two-year-old dogfish, *Mustelus canis,* of both sexes were employed since sufficient numbers of one age group were not obtainable. However, most of the animals were yearlings. The fish were captured either by large traps or caught on hook and line, transferred to large live cars, and fed daily with generous portions of fresh squid. When a sufficient number of fish for a particular experiment had accumulated, they were placed in smaller indoor tanks, marked so that each individual could be identified, and an initial blood sugar determination was made. Further treatment will be described under separate headings in the experimental section.

Pancreatectomy in the dogfish is a simple matter and merits no description. Partial hypophysectomy is a more complicated operation, however. After immobilization of the animal by cooling in iced seawater (Parker, 1937) the fish was strapped to a V-shaped operating board, and the mouth held open by retractors. An incision about one-half inch in length was made in the oral epithelium, exposing the cartilaginous skull directly over the hypophysis. With a small scalpel, a 3 mm. incision was made in the skull parallel to and slightly posterior to

the optic chiasma. Two somewhat longer incisions were then made from the edges of the lateral incision posteriorly and parallel to the sides of the jaws. The flap thus formed was bent caudally until the anterior edge of the neuro-intermediate lobe was visible. For the removal of the anterior lobe alone, the distal part was dissected away from the neuro-intermediate lobe by passing a small curved knife through the connections between the two lobes. The tongue of the anterior lobe was then dissected off the hypothalamus and the entire lobe removed with fine forceps. For the removal of the neuro-intermediate lobe alone, the distal portion of the anterior lobe was dissected free of the neuro-intermediate lobe and deflected slightly. The large neuro-intermediate lobe was then either lifted out, usually in one piece, or removed by suction. For complete hypophysectomy, the procedure was reversed. The tongue of the anterior lobe was removed first, and then the remainder of the pituitary, after severing it from the ventral lobe. Complete hypophysectomy as used here consists of the removal of the anterior and neuro-intermediate lobes. The ventral lobe, of uncertain significance, remained embedded in the cartilage. The sacculus vasculosus was also left intact. When the operation was completed, the cartilagenous flap was allowed to settle in place thus closing the wound, and the oral epithelium sutured. About 4 to 7 minutes are required for hypophysectomy, and about 10 to 15 minutes for pancreatectomy. With assistance both operations can be performed in about 10 minutes.

Blood was obtained from the caudal artery and subsequently analyzed by the macro-precipitation method of Miller and Van Slyke (1936).

Experimental

The Effect of Background on the Resting Blood Sugar

If intermedin is the diabetogenic hormone, it might be expected that fishes subjected to a prolonged adaptation to a black background would have higher blood sugar values than those adapted to a white background, since the former state is produced by a continuous discharge of intermedin whereas the latter state results from an inhibition of the secretion of this hormone.[3] Citation of such a condition in the toad has already been made. Accordingly, dogfishes which had been previously fed for various periods, were brought into the indoor tanks, and an initial

[3] This version of the physiology of the neuro-intermediate lobe in the dogfish is, however, open to criticism (cf. Abramowitz, 1939*b*) since it is believed by some that the white-adapted state is due to the secretion of a new hypophyseal hormone. However this may turn out, it is generally agreed that during black-adaptation there is an abundance of intermedin secretion, and during white-adaptation, less or probably no circulating intermedin.

TABLE I

The effect of background on the blood sugar of starved dogfish.
Blood sugar in milligrams per cent.

Initial	Days Starvation after Initial Determinations							Background	
	1	2	3	4	5	6	7		
216	198	168	127	122	117	132	116		
236	140	131	125	141	128	—	110	Black	
Average 226	169	149	126	131	122	132	113		*A*
252	138	130	113	130	98	136	97		
219	162	123	101	100	94	106	93	White	
Average 235	150	126	107	115	96	121	95		
126	130	146	112	107	101	106	124		
116	147	136	—	120	104	113	75		
140	—	156	136	112	126	114	114	Black	
143	150	155	137	116	—	87	87		
Average 131	142	148	128	114	110	105	100		
102	119	108	98	104	111	102	109		
112	145	148	148	118	124	110	124		
164	150	149	150	132	134	107	104	White	*B*
150	149	155	140	117	121	124	135		
Average 132	140	140	134	118	122	111	118		
156	—	135	121	—	103	—	—		
139	—	125	122	—	137	—	—		
121	—	134	132	—	116	—	—	Intermediate	
137	—	125	161	—	120	—	—		
Average 138		129	134		119				
Grand Average 158±11.3	148±5.5	139±3.8	128±4.3	118±3.4	116±3.3	112±4.0	107±4.7		
No. of Animals (16)	(11)	(16)	(15)	(12)	(15)	(11)	(12)		

blood sugar value obtained. The fishes were then placed on white, black, and intermediate backgrounds, starved [4] for the duration of the experiment, and their blood sugar level followed daily.

As the data in Table I clearly show, there is no effect of background on the resting blood sugar level of the dogfish. In one series (Column A, Table I) the black-adapted fish showed higher values than the white-adapted fish, but in a second series (Column B) the order was reversed. There are, however, several significant observations to be pointed out. The initial values in Table I as well as in the following tables show considerable variation. This is mostly due to the extent of previous feeding, for fish that were freshly obtained may show low or high values, whereas animals which were fed for several weeks always show high initial values. This is clearly illustrated in the data (Table I) where the initial readings for well-fed animals (Column A) are nearly twice those of meagerly fed animals (Column B). However, the blood sugar values fall gradually during starvation and reach a constant level after the third day. The level reached is quite independent of the magnitude of the initial values.

Since there was no effect of background on blood sugar level, all of the individual determinations were averaged and the result expressed as grand averages at the foot of Table I. These averages, therefore, represent blood sugar level as a function of the time of starvation and serve as standard values for comparison with results obtained in other experimental series. It can be seen that after the second day of starvation the standard deviation of the averages assumes a low constant value, while the variation of the initial determinations is considerable, as seen from the large standard deviation. Consequently, the results for animals on which operations were performed cannot be calculated as percentages of the initial blood sugar values before operation because the initial values are not constant. The only valid comparisons are those made in relation to the standard values on the same day of starvation.

The Effect of Hypophysectomy

Orias (1932) concluded that complete hypophysectomy did not affect the blood sugar of the dogfish, since the average value for 8 fish on the second day following hypophysectomy was no different from that previous to the operation. Orias' observations are undoubtedly correct inasmuch as our data (Table II) likewise show no difference between the hypophysectomized and control groups (Table I) on the second day of starvation. However, removal of the pituitary leads to a marked hypo-

[4] In all of the experiments, the animals were starved throughout the course of an experiment.

glycemia (89–88 mg. per cent) at the fourth day of starvation. There is considerable variation, however.

The effect of partial hypophysectomy was determined by removal of only the anterior lobe in 14 animals, and of only the neuro-intermediate lobe in 11 animals. Both sets of animals were followed for a week. The effect of the removal of the anterior lobe (Table III) is very similar to that of complete hypophysectomy. The level reached (96–94 mg. per cent) is not as low as that following complete hypophysectomy, but there is less variation. Removal of the neuro-intermediate lobe (Table IV), however, does not lead to hypoglycemia, and the average values

TABLE II

The effect of the removal of the pituitary on the blood sugar of starved dogfish. Blood sugar in milligrams per cent.

Initial	Days starvation following operation						
	1	2	3	4	6	9	11
108	—	101	—	72	53		
190	—	99	—	97	89	90	82
216	—	201	—	161	158	129	121
201	—	115	—	98	110	88	
290	—	78	—	63	82		
117	—	270	—	126			
228	—	190	—				
151	102	87	83	70	93		
132	131	117	109	114	75		
177	—	112	—	101	103		
121	—	104	—	35			
112	—	—	—	42	30		
Ave. 170	—	134±17.2	—	89±10.8	88±11.3		
No. of Animals	—	(11)	—	(11)	(9)		

for this series are remarkably close to those of the control series (Table I). The results of the three series of operations as compared with the control series are summarized in Table V.

It can be concluded that the removal of the anterior lobe produces the same effect as that seen after complete hypophysectomy and that the ablation of the neuro-intermediate lobe has no effect on blood sugar. Furthermore, the hypoglycemia, which follows the removal of the anterior lobe, occurs in spite of a continuous secretion of intermedin (Abramowitz, 1939*b*), and on the other hand, hypoglycemia does not occur after ablation of the neuro-intermediate lobe which deprives the dogfish entirely of intermedin.

Table III

The effect of the removal of the anterior lobe of the pituitary on the blood sugar of starved dogfish. Blood sugar in milligrams per cent.

Initial	Days starvation following operation				
	1	2	3	4	6
124	—	106	—	126	85
168					
118	—	107	—	89	89
240	—	116	—	105	110
137	—	165	—	88	—
111	—	114	—	110	121
155	—	103	—	90	115
260					
130	—	88	—	64	32
163	168	112	131	117	113
132	132	113	100	83	
126	—	122	—	93	110
112	—	118	—	94	71
107	—	118	—	90	90
Ave. 148	—	115±5	—	96±4.5	94±8
No. of Animals	—	(12)	—	(12)	(10)

Table IV

The effect of the removal of the intermediate lobe of the pituitary on the blood sugar of starved dogfish. Blood sugar in milligrams per cent.

Initial	Days starvation following operation					
	1	2	3	4	5	7
117	—	193				
154	—	166	110	143		
125	—	109	107	98	115	107
161	—	164	128	117	110	187
122	—	163	111	104	124	141
128	—	130	109	109	—	
134	186	111	113	106	112	
102	114	113	122	110	144	
128	—	124	112	—	114	
132	—	116	89	—	95	
130	—	145	114	—	119	
Ave. 130		139±8.2	112±3.0	112±5.2	117±4.6	
No. of Animals		(11)	(10)	(7)	(8)	

The experiments just described furnish good evidence that the presence or absence of intermedin is without effect on the resting blood sugar level. The most crucial test of the identity of the diabetogenic and chromatophorotropic hormones of the pituitary, however, is one performed on the depancreatized animal. If the continued presence of intermedin in the body is not accompanied by an aggravation of experimental diabetes, and if its complete absence is not accompanied by an amelioration of diabetes, there can be little doubt that intermedin is not the diabetogenic factor. Therefore, the effects of background and of partial hypophysectomy on the blood sugar level of depancreatized dogfish were determined. The results of this series of experiments follow.

TABLE V

Summary table showing the effects of partial and complete hypophysectomy on the resting blood sugar level of starved dogfish.

Operation	Initial value	Blood Sugar in Milligrams Per Cent			
		Days following operation			
		2	4	5	6
Controls	158	139	118		112
Complete hypophysectomy	170	134	89		88
Anterior lobe removed	148	115	96		94
Intermediate lobe removed	130	139	112	117	

Pancreatic Diabetes

Twenty-five fish, fed for various periods of time, were depancreatized after an initial determination had been obtained. Nine were placed in white and sixteen in black tanks. Both sets were starved and the blood sugar values determined daily. The results (Table VI) show that the severity of diabetes is not influenced by the relative amounts of circulating intermedin. In some instances, the black-adapted group showed a slightly more intense diabetes, and in other instances, the reverse was true. All the data were therefore averaged, and the values, given at the foot of Table VI, indicate merely the blood sugar level of depancreatized animals on various days of starvation. The depancreatized animals show values roughly three times those of the control series.

An interesting point with regard to the effects of pancreatectomy in the dogfish is the fact that the severity of the resulting diabetes is inde-

pendent of the blood sugar level prior to the operation. Initial values around 200 mg. per cent indicate fish which had been fed for several weeks prior to operation, yet the resulting diabetes following pancreatectomy is approximately the same as that of poorly fed animals. For example, the initial value of 98 mg. per cent (poorly fed fish) rose to 422, 473 and 500 mg. per cent, a level which is as high as that found in the well-fed individuals. This illustrates again that the results cannot

TABLE VI

Pancreatic diabetes in dogfish starved after initial readings.
Blood sugar in milligrams per cent.

Initial	Days Starvation after Pancreatectomy									
	1	2	3	4	5	6	7	8		Background
193	235	208	255	195						
175	275	210	270	242	191	175				
212	319	291	346	442	508	514	583	496	410	
156	272	172	175	242						
215	371	381	512	584						
269	377	294								White
231	282	245	226							
284	380	342	593							
232	452	330	392	393						
Average 213	320	275	347	350						
149	—	—	250							
198	—	—	216							
209	—	—	150							
234	391	—	392							
217	356	388	—							
98	422	473	500	445						
134	356	418								
135	562	258								
125	—	—	130							Black
236	—	—	432							
197	—	—	280							
146	—	—	400							
160	—	—	350							
198	—	—	526							
Average 174	417	384	330							
[120	94	44]								
[94	29	30]								
Grand Average 191	361±22	308±24.2	337±30	363±50						
No. of Animals (23)	(14)	(13)	(19)	(7)						

be calculated in terms of the initial values. Secondly, there appears to be no uniformity among the individual fish during the course of their diabetic condition. Some of the animals become progressively more diabetic, some maintain a constant level, and some tend to show decreasing hyperglycemias after the first twenty-four hours.

Inspection of the values listed in Table VI reveals a considerable individual variation. This is somewhat contrary to the results obtained by Orias whose nine depancreatized animals had values between 350 and 431 mg. per cent with an average of 402 mg. per cent at 48 hours fol-

lowing operation. Our results can be arbitrarily classified into four main groups: (1) severe diabetes, (2) mild diabetes, (3) no diabetes, and (4) hypoglycemia. The first group represents values above 300 mg.

TABLE VII

Effects of complete and partial hypophysectomy on pancreatic diabetes. Blood sugar in milligrams per cent.

Pancreas + Pituitary removed	Pancreas + Anterior lobe removed	Pancreas + Intermediate lobe removed
100	[96	[96
110	79	28]
117	43	
133	88]	
176		
173		175
134		182
	159	120
	146	126
	121	196
368	120	164
350	114	
370	143	
311	119	
425		800+
437		391
		385
	335	335
	433	484
212	382	548
233	333	682
289	462	307
213		478
292		319
282		445
293	285	689
273	284	392
204	228	
247	206	
294	202	
212	298	284
224	233	297
	219	254
		226
		211
Ave. 249±18	241±23	354±36
No. of Animals (26)	(20)	(24)

per cent, the second between 200 and 300 mg. per cent, the third between 100 and 200 mg. per cent, and the fourth, values below 100 mg. per cent which is below that of a normal animal. Such a classification is, of

course, quite rough, but it serves to bring out a significant comparison with results obtained in the next section where both pancreas and pituitary were removed. The percentages of the animals falling into the various groups are shown in Table VIII. Most of the depancreatized animals (56 per cent) become severely diabetic, 24–28 per cent are only mildly diabetic, and 8–12 per cent do not develop hyperglycemic levels at all. That a small percentage of animals does not become diabetic seems to be a constant factor, for a similar percentage has been found in all cases involving removal of the pancreas. Two of the twenty-five animals, or 8 per cent, developed hypoglycemia. These two animals were not included in the calculations of the averages in Table VI, since they are obviously anomalous. This, too, appears to be a constant factor for it has recurred in the other series of operations in about the same percentage of cases.

TABLE VIII

Summary table showing distribution of results among arbitrary classes of diabetes. Figures indicate percentage of the animals comprising the various types.

Operation	Hypoglycemia	No Diabetes	Mild Diabetes	Severe Diabetes
Range of blood sugar, Mg. %	<100	100–200	200–300	>300
Pancreas alone removed	8%	8–12%	24–28%	56%
Pituitary + pancreas removed	0–3.85%	23.2–26.8%	50%	23.2%
Anterior lobe + pancreas removed	16.6%	29.4%	33.6%	20.4%
Intermediate lobe + pancreas removed	7.7%	23.1%	19.2%	50.0%

A few animals were followed immediately after pancreatectomy to determine how soon hyperglycemia appeared. The blood sugar rose shortly after operation and reached peak levels within nine to twelve hours.

The Effect of Complete and Partial Hypophysectomy on Experimental Diabetes

Seventy-six animals were depancreatized and hypophysectomized simultaneously. In 26 animals, the pancreas and the whole pituitary were removed; in 26 the pancreas and only the neuro-intermediate lobe were removed; and in 24 the pancreas and only the anterior lobe were removed. Blood sugar analyses were made daily, but due to high mortality, only the determinations on the first day following operation were listed in Table VII. An individual variation in the severity of diabetes, such as that described for pancreatectomy, was encountered, and the per-

centage of cases showing the various types of diabetes is given, for each of the 3 series of operations, in Table VIII.

Complete hypophysectomy tends to reduce the severity of experimental diabetes. As shown in Table VII, the average blood sugar level is 249 mg. per cent, which is somewhat lower than the average of 9 animals (281 mg. per cent) found by Orias. The greatest number of animals (50 per cent) show a mild type of diabetes, and 23 per cent show either no diabetes or a severe diabetes. None of the animals was hypoglycemic at the first day, but 2 animals showing the lowest readings (100 and 110 mg. per cent) became hypoglycemic on the third and fourth days.

The average value of 20 animals from which both the anterior lobe and the pancreas were removed was 241 mg. per cent, which is practically the same level as that found in completely hypophysectomized-depancreatized animals. Sixteen per cent showed hypoglycemia and was not included in the calculation of the average for this group. Twenty-nine per cent showed no diabetes and 20 per cent showed a severe type. On the whole, the results of this group are similar to those obtained when the pancreas and the entire pituitary were removed.

The results obtained when only the neuro-intermediate lobe and pancreas were removed are quite different from those of the other two groups of animals in this series of operations but similar to those obtained when only the pancreas was removed. The average value of this group (excluding the 8 per cent showing hypoglycemic levels) was 354 mg. per cent. Most of the animals (50 per cent) showed severe diabetes, 19 per cent showed mild diabetes, and 23 per cent were not diabetic. One of the animals showed an extremely high level of 800 mg. per cent, which is probably an anomalous value. Excluding this value, the average of this group would be somewhat lower (334 mg. per cent), which is still a severely diabetic level.

Examination of the summary table (Table VIII) leaves little doubt that complete hypophysectomy alleviates pancreatic diabetes. Anterior lobe removal accomplishes the same result but the removal of the neuro-intermediate lobe has little effect on the severity of experimental diabetes. These results are consistent with those of the previous experimental series, and lead to the conclusion that the diabetogenic hormone in the dogfish is produced by the anterior lobe of the pituitary, and that this hormone is not identical with intermedin.

Summary

The resting blood sugar level and the severity of pancreatic diabetes are not influenced by fluctuations in intermedin secretion in the dogfish.

Complete hypophysectomy is followed by the appearance of hypoglycemia on the fourth day following the operation, a response which is also produced by the removal of the anterior lobe alone. Removal of the neurointermediate lobe does not affect the resting blood sugar level. Complete hypophysectomy ameliorates the severity of pancreatic diabetes, as does the removal of the anterior lobe alone. The removal of the neurointermediate lobe alone does not influence pancreatic diabetes. These results show that intermedin and the diabetogenic hormones of the pituitary of the dogfish are not identical.

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